

B-Cell Maturation

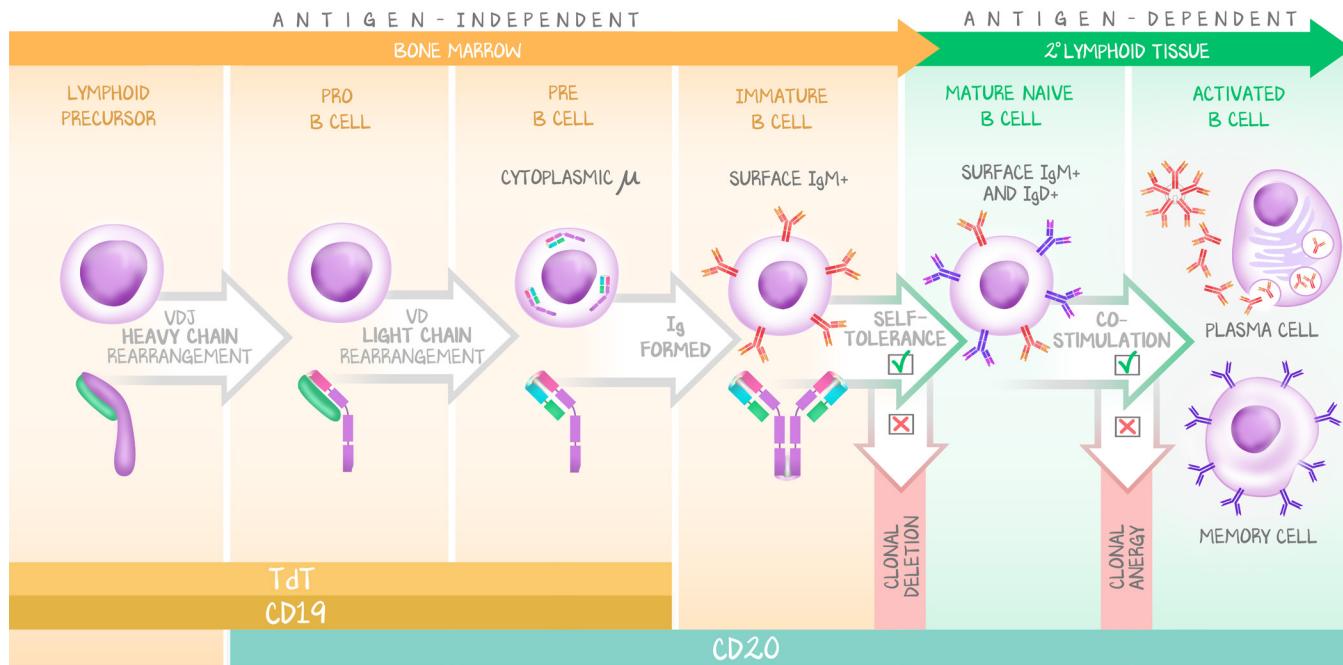
Introduction and Overview

B cells come from bone marrow. They grow there. They mature there. B cells express immunoglobulin. Mature naive B cells and memory cells make membrane-bound surface-protein immunoglobulin. Activated B cells and plasma cells make secreted immunoglobulins called antibodies. Remember that immunoglobulin is the same thing in both places, but one (surface protein) is attached to the cytoplasm by the Fc portion and one (the antibody) has its Fc portion exposed (unbound to the cell surface).

Intact complete immunoglobulins consist of two monomer-halves, each monomer-half consisting of a heavy chain and a light chain. That monomer-half is called **cytoplasmic μ** . B-cell maturation is defined by its progression from making only a primordial pre- μ structure to making intact immunoglobulin.

The immunoglobulin needs to be made and tested. B-cell maturation occurs in the bone marrow, which is **antigen-free**. And since B cells become the antibody-secreting cells, and antibodies are the most specific means of an immune defense, there must be a mechanism that allows for nearly infinite permutations of immunoglobulin construction. This is called **receptor diversity**—it's spontaneous, and occurs without antigens to drive it. That allows any B cell to recognize any antigen. But it also means that it's possible that a B cell makes an immunoglobulin that would mistakenly identify self as foreign. Therefore, the process of maturation must involve some mechanism to ensure **self-tolerance**. **Self-tolerance** simply means that our own immune system should not react against self. If a B cell identifies self as foreign, its cell line is eliminated, a process called **clonal deletion**. There is a second mechanism to ensure tolerance to self called **clonal anergy**, discussed in lesson #8: *B-Cell Activation*.

An **idiotype** is the antigen-binding receptor region's shape on an immunoglobulin—the thing that responds to pathogens. And because the immune system must respond to potentially millions of pathogens, there must exist a means of generating as many idiotypes of antigen receptors as possible. Coding every single different idiotype on DNA would be impossibly wasteful. Instead, what happens is that the DNA consists of a series of segments that can be variably rearranged, producing near-infinite possible idiotypes from a single strand of DNA.

**Figure 7.1: Maturation of B Cells**

The heavy-chain VDJ rearrangement (IgH) and subsequent light-chain VD rearrangement allow for the production of the μ half-monomer. Two μ proteins together form the intact immunoglobulin.

Immunoglobulins have a constant and a variable region. The **constant portions** of the heavy and light chains are called constant, because . . . well . . . they stay the same. And the **variable portion** of the heavy chain and the **variable portion** of the light chain are called variable because . . . they're variable. “Variable” means “randomly rearranges DNA segments to get any one permutation for this particular immunoglobulin.” The variable portion is the area that binds to antigen, so that variability is essential to create the diversity needed to recognize the infinite different types of antigen that one might encounter.

B-Cell Maturation and Receptor Diversity

The B cell starts off as a **progenitor cell** without a heavy chain, a light chain, or any surface protein or immunoglobulins. It has a **primordial structure** that'll eventually become the half-monomer, cytoplasmic μ .

A pluripotent progenitor differentiates into the B lineage as a **pre-pro-B cell**. It acquires the “B-cell things” but does not express anything regarding μ or an immunoglobulin. The “B-cell things” are **CD19**, **CD20**, and **TdT**. TdT is the marker of “young” or “immature” B cells. Once an immunoglobulin receptor is expressed on the surface, the B cell is no longer “young” and no longer expresses TdT.

As the B cell begins to mature, in order to be considered in the **pro-B-cell phase**, there must be **heavy-chain rearrangement**. The heavy-chain variable region is made of **3 recombined DNA segments**—the **variable (V)**, the **diversity (D)**, and the **joining (J)**. The heavy chain is made up of a combination of VDJ; that is, a random V, a random D, and a random J. From the primordial

nothingness, the recombination of VDJ leaves the pro-B cell expressing what'll be the heavy chain of all its immunoglobulins. At this point, the heavy chain is ready, but the light chain hasn't been touched. It's too early for this cell to do anything; the chains are still too undeveloped to be a real protein. It still has the “B-cell things” (CD19, CD20) and it is still “young” (TdT), but the only thing expressed is a primordial cytoplasmic μ .

To cross into the **pre-B-cell phase**, the same thing happens to the light chain: **light-chain rearrangement**. Same concept, only the light-chain variable portion has only two types of gene segments—VJ. The light chain is made by combining a random V and a random J. Having finished the heavy chain as a pro-B, and having finished the light chain as a pre-B, we have the thing that'll become an immunoglobulin. But pre-B hasn't put the monomer-halves together, so all the pre-B cell expresses is a **cytoplasmic protein** called the μ **protein**. There's no immunoglobulin yet. No membrane-bound surface protein. It still has the "B-cell things" (CD19, CD20), and it is still "young" (TdT).

To become an **immature B cell** the cell learns how to take the **mu (μ) proteins** and stick them together, to make a complete **immunoglobulin**. It takes the heavy-chain-light-chain monomer-half (2 chains each: 1 heavy, 1 light) and disulfide bonds it to another heavy-chain-light-chain monomer-half. This completes the monomer immunoglobulin, the Y-shaped thing that acts as membrane-bound surface protein or can be secreted as antibody. The immature B cell, having made an immunoglobulin, now expresses the immunoglobulin as the membrane-bound **surface immunoglobulin IgM**. Expressing an immunoglobulin receptor, it is no longer "young" (no TdT) but is still a B cell so has "B-cell things" (CD19, CD20). We learned that IgM, when in circulation as an antibody, forms a pentamer, connected by a J chain at the Fc portion of the immunoglobulin. But at this phase in B-cell maturation, the Fc portion of the IgM immunoglobulin is attached to the membrane of the B cell. No secretion of antibody occurs.

This cell can't be released from the marrow yet. Only a **mature naive B cell** can be released from the bone marrow. A mature naive B cell is **surface IgM⁺**, but it's also **surface IgD⁺**. IgD is both a "ticket to ride"—proof that the B cell passed the bone marrow's test and is ready for entry into a secondary lymphoid organ—and a probationary pass, a kill-switch fail-safe. Should this mature naive B cell identify an antigen as foreign, but the mature T cell supervising it doesn't, then that B cell is sent to anergy (and ultimately dies). Clonal anergy is discussed in greater detail in the next lesson.

The fate of the mature naive B cell is to become inert (IgD surface protein), be activated into plasma cells (immunoglobulin-secreting cells), or be transformed into a memory cell (IgG surface antibody). This is discussed in detail in #8: *B-Cell Activation*.

The Functional Consequences of Receptor Diversity

Because the process of immunoglobulin formation is antigen-free, receptor diversity is necessary to attempt to cover all pathogens that could be encountered. Across all the B cells that ever mature, each one a random combination of light chain recombination and heavy chain recombination, the number of permutations approaches a number greater than humans can conceive (figuratively infinite). Literally, the variable and constant domains change to produce many (infinite) permutations of immunoglobulin structures. Some of those permutations will result in the ability to fight some antigen. But some of those permutations may result in an immunoglobulin that has too high affinity for self. This loss of tolerance is what causes autoimmune disease (#14: *Autoimmunity*). While random variation must be permitted to ensure that our immune system can recognize any possible pathogen, the bone marrow must also install mechanisms to prevent self-reactivity. There are two fail-safe mechanisms. First, if the **bone marrow** detects **high self-affinity**, then the cell line is never allowed to leave the marrow. The cell is killed, and its line eliminated, a process called **clonal deletion**. Second, the mature naive B cell is allowed to leave the marrow once it has escaped clonal deletion and is tagged with IgD. The mature naive B cell has both surface IgM⁺ and surface IgD⁺. If the B cell identifies antigen but fails to receive a costimulatory signal from T cells, the default is to undergo **clonal anergy**. Anergic B cells lose IgM surface proteins and express only IgD, an impotent immunoglobulin, and one that fails to receive growth signals, so by default, dies off. If the B cell is activated by T cells, IgD is lost.

The second consequence of this random rearrangement is that any immunoglobulin produced by a plasma cell expressing IgM is likely to have **poor antigen specificity** for any one pathogen—the B cell's IgM receptor had not yet seen any antigen yet. The random combination is unlikely to develop an antigen-binding site that is specific for exactly one pathogen. The bone marrow just makes so many versions of immunoglobulin that it will “cover” every pathogen it could encounter. But it covers every pathogen only very weakly (low affinity). Once activated, that particular cell does two things. It makes immunoglobulins that are poorly specific for that antigen (IgM) to get things started, called the initial antibody response. But the cell also begins proliferating. All the cells rapidly proliferating fight over the antigen; the one with the highest affinity for the antigen receives the proliferation signal. The ultimate response to that initial recognition of a new antigen is to create cells that have modified their variable regions to now be very specific against that antigen. These cells can identify the antigen on repeat exposure and produce cells that make antibodies against that antigen.

The random permutation in the bone marrow makes receptors that are just random. But if the B cell recognizes an antigen, that randomly generated combination is replaced with a super-specific variant.

Other Markers of B Cells

B cells can be identified by their CD markers, present from lymphoid precursors through memory and plasma cells. **CD19**, **CD20**, and **TdT** are markers of B cells. B cells have “double-digit CD markers” while T cells have “single-digit CD markers.” A quick trick to help you keep them straight.

TdT is expressed in all B cells from lymphoid precursor to the point of immature B cell. This is only relevant in the sense that malignant transformation of lymphocytes can be detected by the presence of TdT, if that malignancy is a blastic early cancer called acute lymphoblastic leukemia (ALL). TdT is CD10. TdT is also expressed on T-cell lymphoblasts, and just as it can be used to identify early ALL, it can be used to detect lymphoblastic leukemia and lymphoblastic lymphoma. Therefore, as you can see, these cell markers are not just numbers to memorize; they have a clinical relevance.